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Genic introgression from an invasive exotic fungal forest pathogen increases the establishment potential of a sibling native pathogen

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Abstract

Significant hybridization between the invasive North American fungal plant pathogen *Heterobasidion irregulare* and its Eurasian sister species *H. annosum* is ongoing in Italy. Whole genomes of nine natural hybrids were sequenced, assembled and compared with those of three genotypes each of the two parental species. Genetic relationships among hybrids and their level of admixture were determined. A multi-approach pipeline was used to assign introgressed genomic blocks to each of the two species. Alleles that introgressed from *H. irregulare* to *H. annosum* were associated with pathways putatively related to saprobic processes, while alleles that introgressed from the native to the invasive species were mainly linked to gene regulation. There was no overlap of allele categories introgressed in the two directions. Phenotypic experiments documented a fitness increase in *H. annosum* genotypes characterized by introgression of alleles from the invasive species, supporting the hypothesis that hybridization results in putatively adaptive introgression. Conversely, introgression from the native into the exotic species appeared to be driven by selection on genes favoring genome stability. Since the introgression of specific alleles from the exotic *H. irregulare* into the native *H. annosum* increased the invasiveness of the latter species, we propose that two invasions may be co-occurring: the first one by genotypes of the exotic species, and the second one by alleles belonging to the exotic species. Given that *H. irregulare* represents a threat to European forests, monitoring programs need to track not only exotic genotypes in native forest stands, but also exotic alleles introgressed in native genotypes.

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Keywords
Biological invasions, fungi, *Heterobasidion*, hybridization

Introduction

Introgression, i.e. the exchange of genetic material between interfertile species through hybridization, is recognized as a significant catalyst of evolution of eukaryotes (Arnold et al. 2008; Edelman et al. 2019). Thanks to increasing world trade, to advances in genetic characterization and to the democratization of genomic research technologies, the frequency and estimates of hybridization events across all phyla have risen exponentially in recent years (Rieseberg et al. 2000; Barton 2001; Seehausen 2004; Mallet 2005; Le Gac and Giraud 2008; Dasmahapatra et al. 2012; Clarkson et al. 2014).

Although the interspecific transfer of genetic material can be a stochastic process not necessarily involving genes responsible for phenotypic adaptive variation (Suarez-Gonzalez et al. 2018), there are many cases in which introgressive events have been documented to provide novel genes and alleles or to generate favorable allelic combinations that confer adaptive advantages to the recipient organism (Suarez-Gonzalez et al. 2018). Introgression may also increase the fitness of hybrids, allowing them to be competitive against parental species, particularly in novel ecological niches (Mallet 2007), thus facilitating successful invasions (Schierenbeck and Ellstrand 2009; Lee 2002). In plants, it has been well documented that hybridization may result in evolutionary changes increasing invasiveness (Schierenbeck and Ellstrand 2009; Abbott et al. 2003). It is also recognized that hybridization can lead to the emergence of new pathogenic fungal species (Brasier 2000, 2001; Fisher et al. 2012; Giraud et al. 2008; Gladieux et al. 2014; Stuckenbrock 2016).

Stukenbrock (2016) suggested that studies integrating genomic and experimental data are pivotal to understand the evolution of fungal hybrids in nature. Whereas fungal hybrid genotypes can be generated and studied *in vitro* (Olson and Stenlid 2001; Giordano et al. 2018), hybrids resulting from natural selection are difficult to find in nature, given that only a few cases of ongoing hybridization are known (Garbelotto et al. 1996; Gonthier and Garbelotto 2011; Hughes et al. 2013; Pryszcz et al. 2014; Menardo et al. 2016; Sillo et al. 2019).

The fungus *Heterobasidion irregulare* Garbel. & Otrosina is a pathogen of pines in North America that was accidentally introduced into central Italy in 1944, during World War II (Gonthier et al. 2004). The pathogen has become invasive, (Gonthier et al. 2014), and is now included in the A2 list of organisms recommended for regulation by the European and Mediterranean Plant Protection Organization (EPPO 2015). In Central Italy, where *H. irregulare* has been reported so far, its Eurasian sister species *H. annosum* (Fr.) Bref. is also present; however the invasive exotic species is significantly more abundant than the native one (Gonthier et al. 2007). The invasiveness of the exotic species has been experimentally shown to depend not on pathogenicity, which is similar between the two species (Garbelotto et al. 2010), but on its greater ability

to saprobically colonize wood and to produce more fruiting bodies, thus increasing its sporulation rate compared to the native sister species (Giordano et al. 2014). Primary infection by *Heterobasidion* is indeed effected by basidiospores colonizing exposed woody surfaces of stumps and wind-thrown trees (Garbelotto and Gonthier 2013), hence an enhanced ability to saprobically colonize wood would confer significant advantages to individuals carrying them. Genomic analyses have corroborated the above hypothesis by showing that genes involved in saprobic growth are significantly more divergent between the two species than expected by genetic drift, while, on the contrary, genes involved in pathogenicity appear to be rather conserved between the two, having undergone purifying selection (Sillo et al. 2015). A recent phenotypic and transcriptomic characterization of artificial *Heterobasidion* hybrids has also determined that *H. irregulare* nuclear genes related to nucleus-mitochondrion communication may confer an advantage to hybrid genotypes compared to *H. annosum* genes involved in the same function (Giordano et al. 2018).

The two species have evolved allopatrically for 34–41 millions of years, but have remained interfertile (Garbelotto and Gonthier 2013). Their recent sympatry has resulted in one of the few known current examples of fungal hybrid swarms (Gonthier and Garbelotto 2011). A study of over 260 genotypes from the hybrid zone in Italy using AFLP and multi gene phylogenies has indicated that, depending on site, up to 42% of the genotypes had an admixed genome, with levels of genomic admixing varying between 5% and 45% (Gonthier and Garbelotto 2011). Sequencing of 11 loci has indicated that in 85% of cases, introgression involved alleles of one species being absorbed by the other, while intragenic recombination was detected only in 15% of cases (Gonthier and Garbelotto 2011). Thus, it is expected that the introgression of whole alleles, and not recombination, may be the dominant process reshaping genomes through hybridization between *Heterobasidion* species. This study may confirm or disprove such expectations.

Two studies using different genetic markers (Gonthier and Garbelotto 2011; Garbelotto et al. 2013) have shown that there is no significant genetic structuring within the invasive and the native species (Gonthier et al. 2014, 2015). Thus, despite the relatively large area of the hybrid zone, it can be assumed that a single population of *H. irregulare* is hybridizing with a single population of *H. annosum.* The lack of population structure further simplifies assignment of alleles to either species, given that a genome-wide comparative study has determined that interspecific DNA divergence between the two taxa is high and ranges between 20.0 and 40.0 SNPs/Kbp, while intraspecific divergence within either species ranges only between 1.0 and 4.0 SNPs/Kbp (Sillo et al. 2015).

The model system represented by these interbreeding species with a comparable biology and epidemiology provides a unique opportunity to study the genomics of hybridization and introgression in a natural habitat. The main overarching goal of this study was to investigate a fungal invasion at the gene, rather than at the species level (Petit 2004). Theory predicts that the number of introgression events should be significantly larger from the native into the exotic species, if the latter is in demographic

expansion (Currat et al. 2008; Excoffier et al. 2009). Despite its relatively recent introduction, the exotic fungus is overwhelmingly dominant and its geographic range is increasing, hence, it can be safely assumed that it is in demographic expansion. Our first prediction, thus, is that the number of chromosomal blocks introgressed from the native into the exotic species should be larger than the number of chromosomal blocks introgressed from the exotic into the native species.

Theory predicts that the introgression of alleles between species may be driven by the adaptive advantages such alleles may provide (Clarke et al. 2002; Fitzpatrick et al. 2010). In our case, invasiveness of the exotic species has been associated with genes involved in saprobic wood decay and in nucleus-mitochondrion communication, hence our second prediction is that alleles of genes involved in these two functions would be introgressed from the exotic into the native species, and that, vice versa alleles involved in these two functions would not be lost by *H. irregulare* when hybridizing with *H. annosum*.

Although we have yet to identify *H. annosum* alleles that may provide an advantage to *H. irregulare* genotypes acquiring them, we can hypothesize that alleles of regulatory genes evolutionarily adapted to the Italian landscape, or that genes putatively related to hybrid genome stability may be good candidates as alleles introgressing into *H. irregulare*. The latter group of alleles may enhance the survival of these hybrids by counteracting the effects of genomic instability caused by the large number of introgression events, as has been shown in other cases (e.g. in animals; Walsh et al. 2018),

Finally, our last prediction is that *H. annosum* genotypes that have absorbed *H. irregulare* alleles involved in saprobic decay and nucleus-mitochondrial communication should display an increased saprobic ability when grown on woody substrates. Conversely, given that virulence does not differentiate the two species, there should be no clear association between genomes of hybrids and this trait. Nonetheless, because all genotypes tested in this study were established in nature and thus naturally viable, we predict pathogenicity should be within the range displayed by pure parental genotypes. In addition, *H. irregulare* genotypes that have absorbed a large number of chromosomal blocks from *H. annosum* may display decreased saprobic growth, pathogenicity or both, due to the effects of large-scale structural genomic rearrangements caused by the large extent of genetic introgression from *H. annosum*.

As stated above, the main overarching goal of this study was to investigate a fungal invasion at the gene level, rather than at the species level (Petit 2004). Are exotic alleles introgressed in the native species, and do these alleles confer an adaptive advantage to genotypes of the native species that acquire them? Given that the exotic fungal species is a forest pathogen that has been identified as a serious threat to the European region and that a strict monitoring program has been recommended for the timely detection of the enlargement of its range (Gonthier et al. 2014; EPPO 2015), this gene centric approach may also indicate whether the monitoring should be expanded to include the detection of specific exotic *H. irregulare* alleles as they introgress in native *H. annosum* populations both within and outside the current zone of infestation by the exotic fungus.

Materials and methods

Biological materials and DNA extraction

An extensive sampling of *Heterobasidion* genotypes was performed in 2005–2006 in the invasion area of *H. irregulare* in Italy where *H. annosum* was also present. Single *Heterobasidion* basidiospores were collected on woody spore traps following the sampling method of Gonthier et al. (2007). Subsequently, fungal genotypes were identified through taxon-specific PCR (Gonthier et al. 2007) as belonging to *H. irregulare* or *H. annosum* and genotyped through Amplified Fragment Length Polymorphism (AFLP) (Gonthier and Garbelotto 2011). Among all sampled genotypes, nine haploid homokaryon hybrid genotypes showing different level of genetic admixture based on the previous AFLP genotyping (Gonthier and Garbelotto 2011) were selected for the current study (Suppl. material 4: Table S1). Mycelia of fungal genotypes were grown in 2% malt extract liquid medium at 25 °C for ten days before being harvested. For the harvesting, approximately 200 mg of fungal mycelium were collected using a vacuum pump, freeze dried overnight and ground using glass beads (diameter 0.2 mm and 0.4 mm) in a FastPrepTM Cell Disrupter (FP220-Qbiogene). Total DNA extraction was performed using DNeasy Plant Mini Kit (Qiagen Inc., Valencia, California, USA), following manufacturer instructions. DNA quantification was carried out by using the ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). DNA quality was assessed by using a chip-based microcapillary electrophoresis system (Experion, Bio-Rad Laboratories, Hemel Hempstead, UK).

Sequencing, mapping, and genotype calling

Paired-End (PE) 100bp DNA libraries were prepared for each genotype and sequenced using an Illumina HiSeq2000 platform at the Vincent J. Coates Genomics Sequencing Laboratory (Berkeley, CA, USA). Low quality reads (Q score < 20) as assessed by using FASTQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/) were removed by using Trim-Galore! v. 0.5.0 with default parameters (Krueger F. Trim-Galore!, available at http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). Reads of each genotype were aligned to the reference *H. irregulare* TC 32-1 genome (Olson et al. 2012) available in the JGI database using the BWA-MEM algorithm of bwa aligner v. 0.7.15 (r1140) optimized for 100bp PE reads (Li and Durbin 2009), with default parameters. The output was used to generate mapping files in SAM format, which were converted in BAM format, sorted, and indexed by SAMtools v.1.3 (Li et al. 2009). The SNP calling was performed by using the *mpileup* command of SAMtools v.1.3 and the *call* command of *bcftools* v. 1.10.2 (default parameters). The VCF output files of each genotype were merged into one VCF file which was filtered by using VCFtools 0.1.12b (Danecek et al. 2011) with "--minQ" set to 25 and "--min-meanDP" set to 5. The SNP panels obtained on the six pure genotypes used in the work by Sillo et al. (2015) were also included in the VCF file, for a total of 15 genotypes. In order to prune SNPs affected by linkage

disequilibrium (LD), i.e., to obtain a set of independent SNPs, the software *plink* v1.07 was used. The VCF file containing the SNP panel was first filtered by removing InDels (by using the vcftools command *–remove-indels*) and then converted on *.plink* format by VCFtools. Independent SNPs (unlinked SNPs) were subsequently selected by setting an LD (r2) threshold of 0.8 for SNP pairs through the plink command "*–indep-pairwise*", setting a windows size of 500 bp and a step size of 50. BAM files were submitted to the EMBL database ENA as a project under the PRJEB36378 accession number.

Genetic relationship among genotypes

In order to infer the genetic relationship among genotypes and to determine the level of admixture, a phylogenomic analysis and a PCoA were performed on the distance matrix obtained by the unlinked SNP panel of the above mentioned 15 genotypes, i.e. six pure and nine hybrids. Analysis was carried out by using REALPHY v. 112 (Bertels et al. 2014). Reads of each genotype were aligned to the *H. irregulare* reference genome *via bowtie2* (embedded in REALPHY software), and then were used by REALPHY to infer a bootstrap consensus phylogenetic tree (1000 bootstrap replicates, initial tree BioNJ, model of nucleotides substitution GTR) via PhyML (Guindon et al. 2010). The tree was midpoint rooted. The distance matrix was then used as input for a Principal Coordinate Analysis (PCoA) performed by using R.

Results from phylogenomic and PCoA analyses, combined with the outcomes of reads assignment by the software *sppIDer* (see below), were used to decide the taxonomic status of hybrids, i.e. hybrids showing clustering with *H. irregulare* pure genotypes and showing >50% of their genomes as belonging to *H. irregulare* were considered as "*H. irregulare* hybrids", while hybrids showing clustering with *H. annosum* pure genotypes and showing >50% of their genomes as belonging to *H. annosum* were considered as "*H. annosum* hybrids".

Detection of introgressed regions and identification of putative introgressed alleles

In order to detect regions putatively introgressed from one to the other parental species in hybrid genotypes, i.e. from *H. irregulare* into *H. annosum* and vice versa, we used a conservative approach informed by the outcomes of three distinct analyses, in order to reduce any bias due to the use of any single test for introgression. Tests were done separately for the two groups of hybrid genotypes, that is the group including the five *H. irregulare* genotypes receiving alleles introgressed from *H. annosum*, and the four *H. annosum* genotypes receiving alleles introgressed from *H. irregulare* (see results).

First, the genome-wide four taxon Patterson's D statistics (ABBA/BABA test) was used to detect hybridization events along genomes. The ABBA/BABA test compares patterns of ancestral and derived alleles within four taxa. Equal numbers of sites for each pattern are expected if gene flow is absent. A positive/negative value of D is generally related to an excess of ABBA/BABA sites and might indicate introgression. In

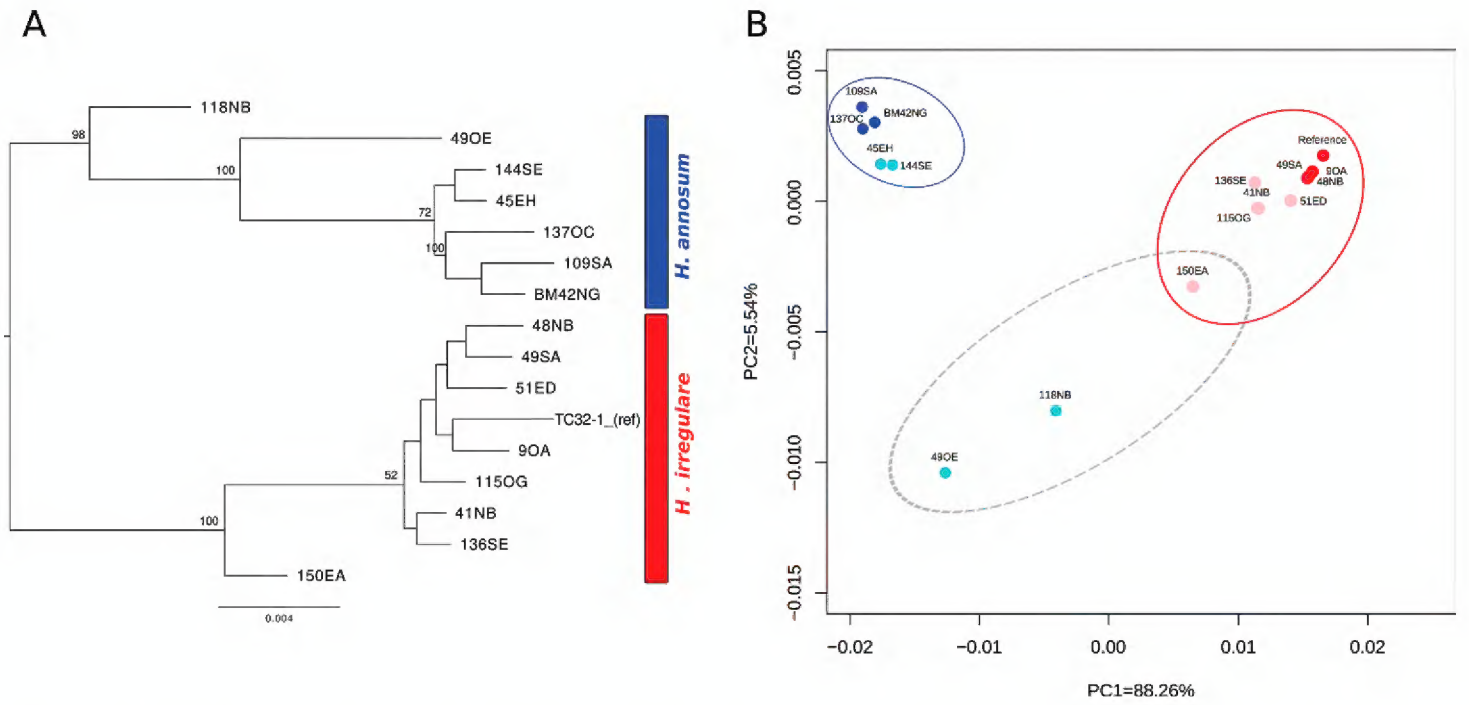

Figure 1. Phylogenomic analysis of the nine hybrid genotypes and PCoA **A** Phylogenomic relationship within reference genome, pure and hybrid genotypes as inferred by REALPHY. Bootstrap values higher than 50 % are showed **B** PCoA on the distance matrix obtained by analysis of unlinked SNPs. Red and blue dots represent pure *H. irregulare* and *H. annosum* genotypes, respectively. Pink and cyan dots represents hybrid genotypes assigned as *H. irregulare* and *H. annosum*, respectively. Clusters were circled as follows: red for *H. irregulare* cluster, blue for *H. annosum*, and gray (dashed line) for F1 hybrids.

detail, the test was performed on each of the 14 scaffolds for each hybrid genotype by *angsd* v.0.700 (http://www.popgen.dk/angsd/) using the following parameters: -doAbbababa 1 -doCounts 1. The parameter *-blocksize* was adjusted according to the dimension in bp of each scaffold. Input files were the BAM files obtained from the alignment of reads against the reference genome. The four-taxon D-statistic test was computed using the following combination: *H. annosum* (P1), hybrid genotypes (P2), *H. irregulare* (P3), and *H. occidentale* (O) as outgroup (*H. occidentale* genotype UC1935443 – Holotype at the Berkeley herbarium - which was sequenced and aligned to the reference *H. irregulare* using the same bioinformatic pipeline performed for the other 15 genotypes). The significance of statistics was assessed using the method of weighted block jackknifing.

Second, reads obtained from the hybrid genotypes were aligned on two reference genomes of the parental species, reconstructed by starting from *de novo* assembly of six pure genotypes published by Sillo et al. (2015). The software *sppIDer* (Langdon et al. 2018) was used to accomplish this task. This software maps the reads of hybrids to a concatenated genome built from the reference genomes of the two parental species, to assess the genomic contribution of *H. irregulare* and *H. annosum* in hybrid genotypes. In order to build the combination reference genome, *de novo* assembly of three pure *H. irregulare* and three *H. annosum* genotypes were used to create two distinct consensus genomes of the two parental species. *De novo* assembly of reads from the three pure genotypes of the two parental species was performed using Velvet v.1.2.08 (Zerbino and Birney 2008) with optimized k-mer = 39 bp, and refined by using IMAGE2 (https://

sourceforge.net/projects/image2/), which was set up at twenty iterations, and used as inputs both unassembled reads and contigs in FASTA format generated by the assembly process (Tsai et al. 2010). The software Contiguator v. 2.7.4 (Galardini et al. 2011) was used to perform the final assembly in order to reconstruct the 14 scaffolds for each genotype. A consensus reference genome for *H. irregulare* and for *H. annosum* was built in order to take into account the intraspecific diversity within species. Indexed VCF files with SNPs of pure genotypes from Sillo et al. (2015) were used as inputs, along with *de novo* refined assemblies, for *bcftools* v. 1.10.2 *consensus* command and a single *consensus* fasta file for each species was obtained. The two consensus genomes were concatenated by using the script *combineRefGenomes.py* embedded in *sppIDer*.

Third, to assess the presence and dimension of introgressed blocks, we used the R package *introgress* v. 1.2.3. (Gompert and Buerkle 2010) on the panel of unlinked SNPs (VCF file) previously obtained. The package was used to generate a graphical representation of SNP ancestry allowing for inspecting the different patterns of introgression. *De novo* assemblies were used to assign the introgressed blocks to one species or the other, in order to quantify both in size and in number the introgression events. Two-tailed T-tests were used to determine the significance of the difference between the average size of introgressed blocks of the two species, respectively, as the Shapiro-Wilk normality test for size of blocks confirmed the normal distribution of this dataset. A Wilcoxon rank sum test (Mann–Whitney U test) was used to assess the significance of difference in the number of introgressed blocks of the two species, as the Shapiro-Wilk normality test for number of blocks confirmed the not normal distribution of this dataset. All tests were performed with R language.

In *H. annosum* hybrid genotypes with different levels of admixture caused by different levels of introgression from *H. irregulare*, regions in scaffolds showing excess of ABBA sites and aligning with *H. irregulare* genome were considered to be regions putatively introgressed from the invasive species to the native. In *H. irregulare* hybrid genotypes, regions in scaffolds showing excess of BABA excess and aligning with *H. annosum* genome were considered to be regions putatively introgressed from the native species to the invasive. Bed files resulting from the alignment of hybrid genotype reads to the concatenated reference genome were used to assign reads to one species or the other, and to extract fasta formatted aligned sequences (through the bedtools v. 2.19 *getfasta* command; Quinlan and Hall 2010). These sequences were searched through *blastn* algorithm (v. 2.2.31) in the gene catalogue of the established manual annotated genome of *H. irregulare* TC32-1 (Olson et al. 2012) with e-value < 0.05 and nucleotide identity > 98%, in order to identify putative genes in the regions assigned to the two different parental species. Related transcripts were identified by ID, and the sequences of their corresponding encoded proteins were analyzed with Blast2GO v.4.1.9 (Conesa et al. 2005) to search for homologs and to determine their Gene Ontology (GO). At the same time, all gene models of the *H. irregulare* reference genome were annotated through Blast2GO v.4.1.9. Fisher enrichment test (False Discovery Rate < 0.05) was performed with Blast2GO v.4.1.9 to search significant differences in frequencies of GO terms compared to all *H. irregulare* gene models (over-representation).

Phenotypic experiment testing virulence and saprobic ability

In order to assess the fitness of hybrid genotypes relative to the fitness of pure genotypes of each one the two species, virulence on host plant and saprobic growth on wood substrate were assessed by means of two distinct phenotypic experiments. All genotypes collected in the invasion area in Central Italy (nine hybrids and four pure genotypes) were used in the assays. For both assays, genotypes were first cultured in Petri dishes filled with Malt Extract Agar (MEA; malt extract agar 33.6 g/L).

The virulence assay was performed by inoculating *P. pinea* germlings, previously grown in sterile conditions, in a microcosm by using a novel and optimized method. Healthy and uniform seeds of *P. pinea* removed from the cones were washed for 10 minutes in running water, the surface was disinfected for 60 minutes in 30% hydrogen peroxide solution, rinsed twice (10 minutes each) with sterile distilled water and deprived of their lignified tegument (free seeds). Subsequently, they were aseptically transferred into glass jars containing 100 mL of water-agar medium (15 g agar, 1 L distilled water). One seed was inserted in each glass jar. After fifteen days at room temperature (25 ± 2 °C), germlings were inoculated by placing on the medium close to their root collar two mycelial plugs (6 mm diameter) obtained from the edges of actively growing fungal cultures. Eight replicates for each fungal genotype were prepared and incubated at room temperature (25 ± 2 °C). As negative controls, eight additional non-inoculated germlings were grown in the same conditions; they remained alive and uncontaminated until the end of the experiment. The germlings were inspected daily for the presence of disease symptoms, including root browning (visible through the agar), needle discoloration, decline in vitality and collapse. The virulence of fungal genotypes was determined on the base of rapidity to death, expressed as the number of days elapsed since pathogen inoculation.

The saprobic assay was performed by measuring the *in vitro* growth rate of each genotype on agar medium mixed with *P. pinea* sawdust to simulate the natural wood substrate as described by Giordano et al. (2018). Sawdust was prepared by cutting a fresh log using a circular saw and collecting the resulting mixture of heart- and sapwood sawdust in a plastic bag. Petri dishes (90 mm diameter) filled with *P. pinea* sawdust and water agar (15 g sawdust, 12 g agar, 1 L distilled water) were inoculated in the center with a single mycelial plug (6 mm diameter) obtained from the edges of actively growing fungal cultures. Ten replicates for each fungal genotype were incubated at 25 ± 2 °C in the dark. The radial growth of fungal cultures was measured every 48 hours for 10 days along two perpendicular lines. Growth curves from the saprobic assay were compared by performing the permutation test based on t-statistics embedded in the *statmod* R package. The number of permutations was set at 100.

To compare the performance of fungal genotypes in both assays, a pairwise distance matrix of measurements between all possible pairs of genotypes was calculated. For the virulence assay, the distance between two genotypes was calculated as the difference between the average number of days elapsed since pathogen inoculation and death of the germling. For the saprobic assay, the distance between two genotypes

was calculated as the difference between the average radial growth at the end of the experiment. Matrices were used as input for Principal Coordinates Analysis (PCoA) performed by using R v.4.0.3 (function *pcoa*).

Results

Sequencing, mapping, and genotype calling

The sequenced reads for each genotype were around 8 million (estimate coverage ranging from 17X to 21X). The proportion of reads that aligned to the reference *H. irregulare* genome ranged from 76.20% to 83.14% in four genotypes (118NB, 144SE, 45EH, 49OE), while in five genotypes (136SE, 41NB, 150EA, 115OG and 51ED) ranged from 89.38% to 91.38% (Suppl. material 4: Table S1).

Phylogenomic and PCoA analyses

The phylogenomic analysis generated a consensus midpoint rooted tree separating two distinct clades, one including pure genotypes of *H. irregulare* and five hybrids (136SE, 41NB, 115OG, 51ED, and 150EA), and one including pure genotypes of *H. annosum* and four hybrids (144SE, 45EH, 118NB, and 49OE).

In the PCoA, *H .annosum* genotypes were distinguishable from *H. irregulare* genotypes by their position along the PC1. However, an additional third cluster was visible within the *H. annosum* cluster, distinguishable from the other two clusters by its position along the PC2. This third cluster included two F1 hybrid genotypes containing comparable levels of the genomes of both species, but still harboring a majority of *H. annosum* SNPs. Genotype 150EA, a putative F1 hybrid harboring a majority of *H. irregulare* SNPs, was in an intermediate position, belonging to both the *H. irregulare* cluster and the cluster including the other two F1 hybrid genotypes.

Detection of introgressed regions and identification of putative introgressed alleles

In order to detect which alleles were consistently transferred between species and to compare introgression levels in the two directions, we focused on the identification of those alleles that were present in all hybrid genotypes belonging to each one of the two groups of hybrids, by intersecting the outcomes of three distinct approaches.

The ABBA/BABA test performed on each scaffold identified an excess of ABBA and BABA sites that can be interpreted as a footprint of introgressive events. On average, an excess of ABBA sites was detected. ABBA/BABA test results across genomes are shown in Figure 2. The excess of ABBA sites was detected in almost all scaffolds, with the exception of scaffolds 2, 7, 12 and 14, suggesting a genome-wide mosaic architecture of hybrid genomes. Interestingly, two blocks important for pathogenic

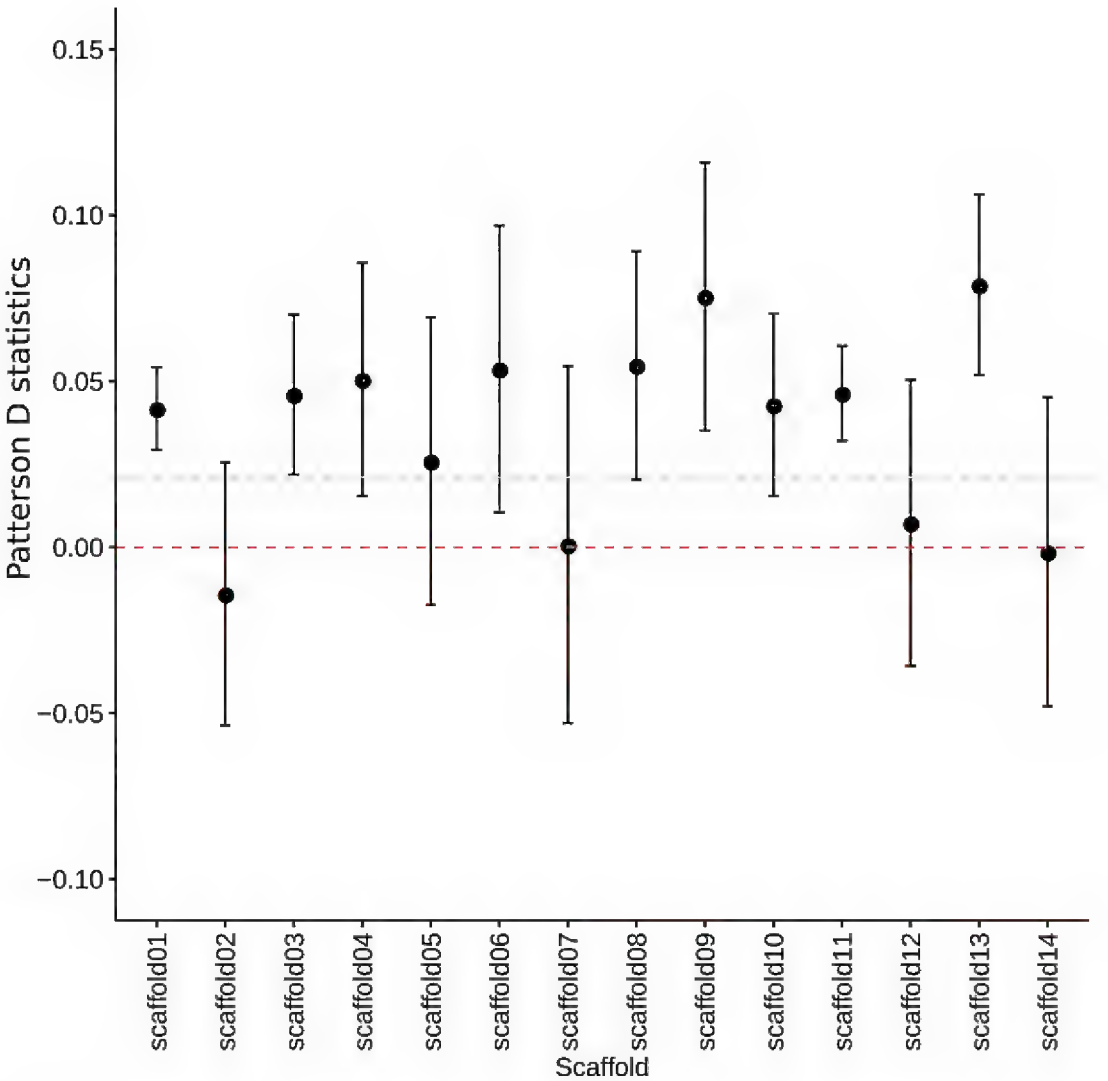

Figure 2. Results of Patterson D statistics (ABBA/BABA test) along scaffolds for all hybrid genotypes. For the four taxon test, population were as follows: *H. annosum* (P1), hybrid genotypes (P2), *H. irregulare* (P3), and *H. occidentale* (O) as an outgroup. ABBA excess D>0 indicate the level of introgression between *H. irregulare* and hybrid genotypes; BABA excess D<0 indicate the level of introgression between *H. annosum* and hybrid genotypes. Gray line represent average D along the 14 scaffolds.

interactions with Norway spruce and Scots pine have been identified in scaffold 12 (Olson et al. 2012).

The *sppIDer* analysis correctly assigned genomic regions of hybrids to one of the two parental species and is visualized in Suppl. material 3: Figure S1. Detailed alignments are reported in Suppl. material 3: Figures S2–10 (a representative example is reported in Figure 3). Thus, this analysis not only identifies the species of origin of all genomic regions in admixed genomes but also confirmed the species assignment of hybrids, based on the percentage of the inherited parental genome (Suppl. material 3: Figure S1). The package *introgress* was used on a panel of 50 559 unlinked SNPs and identified the number and size of introgressed blocks in both directions, i.e. from the native into the exotic pathogen and from the exotic to the native pathogen. (Figure 4). The number of introgressed blocks per genotype and scaffold is reported in Table 1. Introgressed *H. annosum* blocks in *H. irregulare* hybrid genotypes had a maximum size of 7 007 bp and a minimum size of 21 bp (average size in all 5 *H. irregulare* hybrids = 85.04 bp). The total number of introgressed blocks was 14 088. Conversely, *H. irregulare* blocks into *H. annosum* hybrid genotypes had a maximum

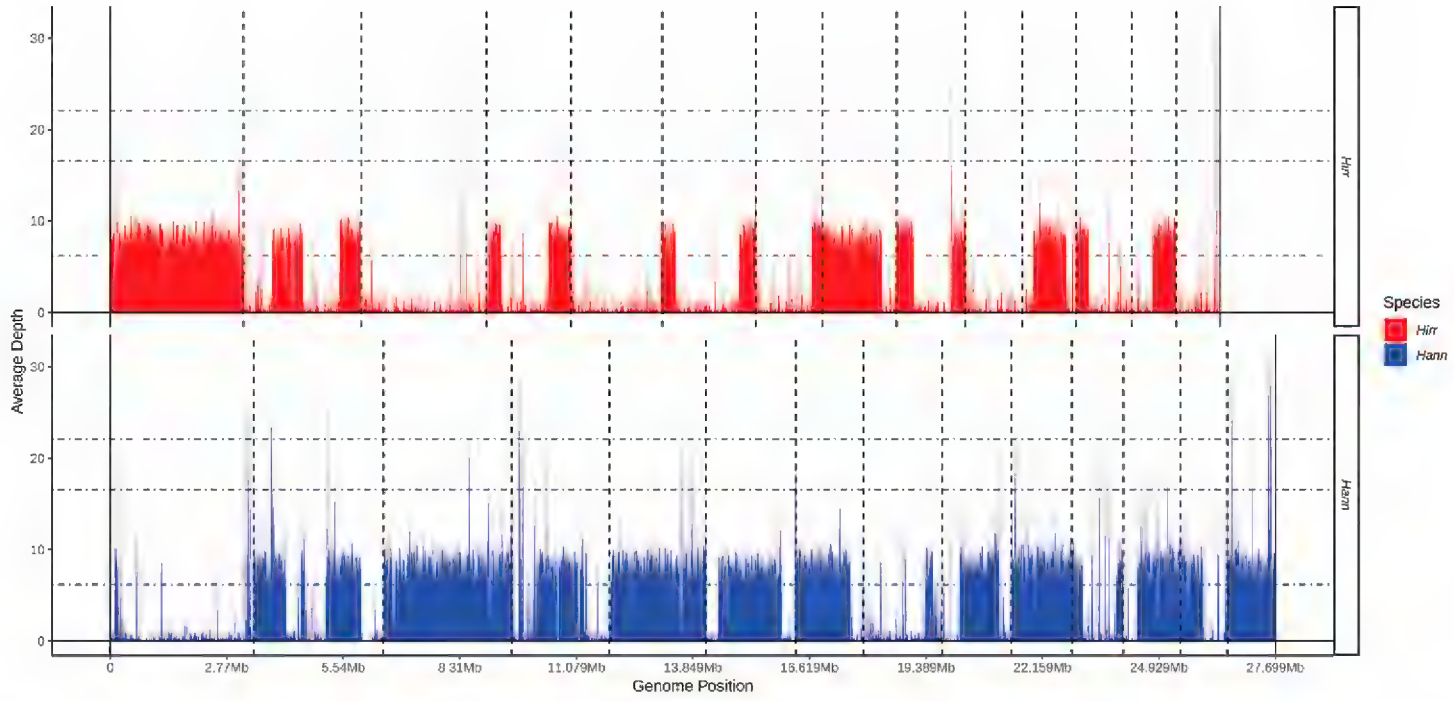

Figure 3. Representative example (hybrid genotype 118NB) of whole genome comparative analysis through *sppIDer*. Average depth coverage of reads after the alignment with the two parental species along the 14 scaffolds of the two genomes.

size of 16 536 bp and a minimum size of 64 bp (average size 890.51 bp), and the total number of introgressed blocks was 5 791. In both cases, some scaffolds showed a low number of introgressed blocks, i.e. 6, 10, 12 and 14 (Table 1). When comparing the size of introgressed *H. annosum* blocks *vs.* the size of introgressed *H. irregulare* blocks, the two were significantly different (two-tailed Student T test p-value $2.2e^{-16}$, t = -63.021, df = 19878). The number of introgressed blocks from one species into the other was also different (Wilcoxon rank-sum test p-value = 0.031746, df = 125, Test statistic W = 19). In summary, *H. annosum* hybrids harbored fewer but larger introgressed blocks than *H. irregulare* hybrids.

In *H. irregulare* and *H. annosum* hybrids, the portions of the genome unaffected by introgression were 2 187 985 bp and 7 255 121 bp in size, respectively. These results suggest that intralocus recombination may be occurring only in a minority of the genome, e.g. within a maximum of 2 Mbp and 7 Mbp in *H. irregulare* and *H. annosum*, respectively. Considering all introgression events in all four *H. annosum* hybrid genotypes, a total of 2 384 introgressed *H. irregulare* alleles were found (Suppl. material 1: Dataset S1). A Fisher enrichment test identified GO terms that were overrepresented in this set, including catabolic pathways, i.e. peroxidases, heterocyclic compound binding, mitochondrial transport and vesicle trafficking (Table 2). Conversely, a total of 1 418 introgressed *H. annosum* alleles were found in *H. irregulare* hybrids (Suppl. material 2: Dataset S2). Fisher enrichment tests on this set determined that overrepresented GO terms were mainly related to nuclear functions including RNA processing, gene expression, nucleotide binding (Table 3). No alleles showing the same GO were ever found to be introgressed in both directions between the two species.

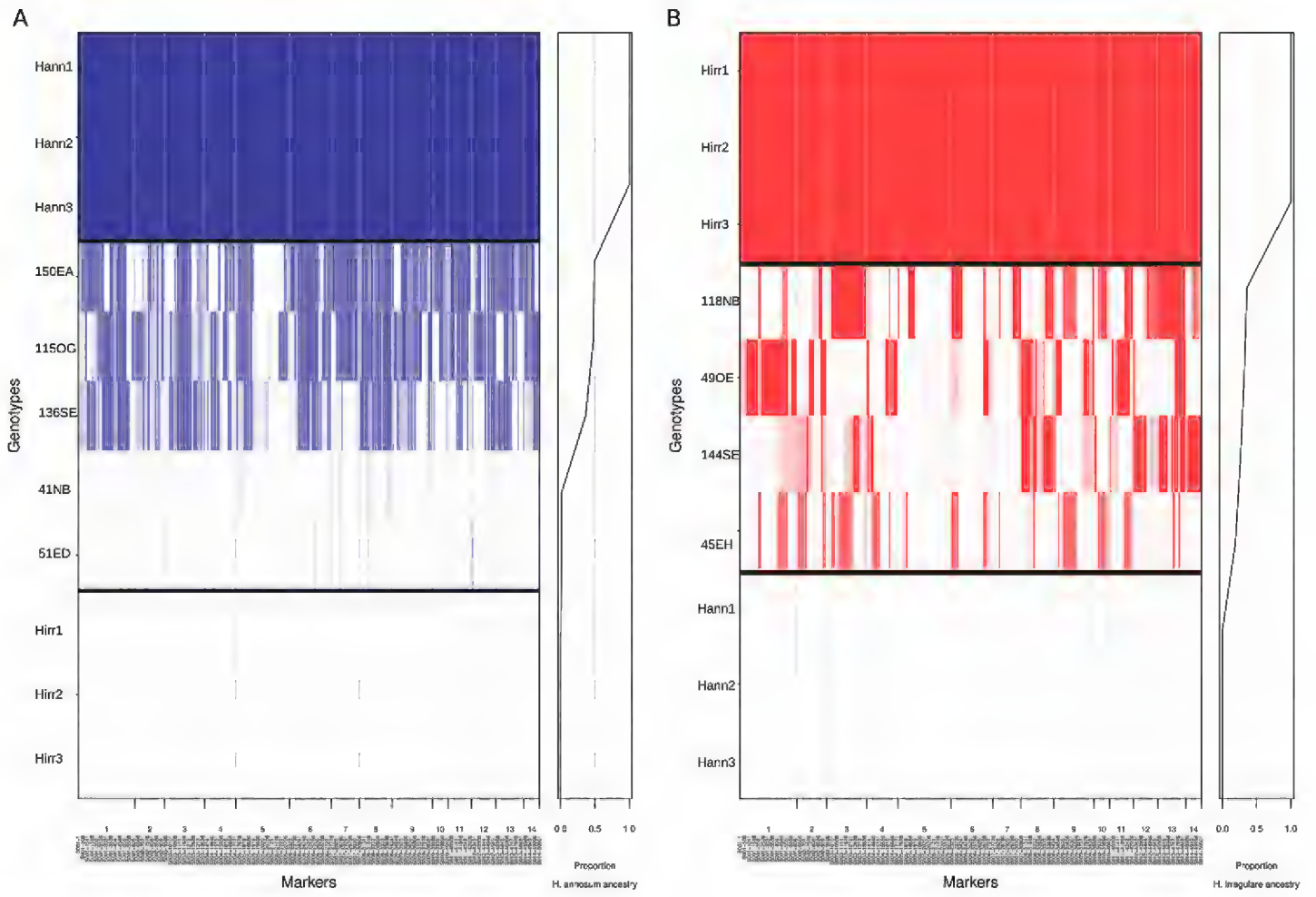

Figure 4. Analysis of introgressed blocks from native to invasive species (**A**) and from invasive to native species (**B**) by INTROGRESS. The dataset of used SNPs (markers) are ordered in x-axis based on genomic locations. In (A), Blue rectangles represent marker assigned to *H. annosum*. Plot on the right represents the fraction of the hybrid genome inherited from *H. irregulare* for each genotype defined as hybrid index. Three pure genotypes of *H. annosum* (on the top) are also included in the analysis. In (B), red rectangles represent marker assigned to *H. irregulare*. Plot on the right represents the fraction of the hybrid genome inherited from *H. irregulare* for each genotype defined as hybrid index. Three pure genotypes of *H. irregulare* (on the top) are also included.

Table 1. Summary of number of introgressed blocks in *Heterobasidion* hybrids.

		Number of introgressed blocks from *H. irregulare* into *H. annosum* hybrids						Number of introgressed blocks from *H. annosum* into *H. irregulare* hybrids					
Scaffold	**ID**	**118NB**	**49OE**	**45EH**	**144SE**	**Sum**	**ID**	**136SE**	**41NB**	**51ED**	**115OG**	**150EA**	**Sum**
scaffold_01		118	50	36	491	695		139	121	90	130	186	666
scaffold_02		254	51	48	202	555		165	249	315	233	274	1236
scaffold_03		243	39	44	47	373		154	281	1171	237	225	2068
scaffold_04		476	33	42	165	716		302	492	161	438	164	1557
scaffold_05		159	35	26	43	263		190	378	73	327	220	1188
scaffold_06		67	25	30	119	241		44	77	320	87	49	577
scaffold_07		204	29	41	72	346		124	226	448	220	53	1071
scaffold_08		209	28	31	189	457		108	152	145	179	272	856
scaffold_09		238	24	26	129	417		118	228	69	243	100	758
scaffold_10		461	31	29	65	586		111	192	188	176	101	768
scaffold_11		178	26	24	136	364		113	206	220	242	109	890
scaffold_12		214	27	26	16	283		82	143	330	157	50	762
scaffold_13		119	23	18	92	252		103	163	499	115	177	1057
scaffold_14		179	22	16	26	243		104	111	91	167	161	634

Table 2. Gene Ontology terms over-represented in the dataset of 2,384 introgressed *H. irregulare* alleles in hybrid *H. annosum* genotypes. FDR: False Discovery Rate (FDR) corrected p-value.

GO-ID	Term	Category	FDR	Sequences (N°) in Test Group	Sequences (N°) in Reference Group
GO:0016209	antioxidant activity	MOLECULAR_FUNCTION	3.405E-03	240	586
GO:0003824	catalytic activity	MOLECULAR_FUNCTION	6.360E-13	996	2531
GO:0043169	cation binding	MOLECULAR_FUNCTION	3.305E-02	147	351
GO:0030130	clathrin coat of trans-Golgi network vesicle	CELLULAR_COMPONENT	2.151E-02	136	314
GO:0051234	establishment of localization	BIOLOGICAL_PROCESS	1.651E-04	214	479
GO:1901363	heterocyclic compound binding	MOLECULAR_FUNCTION	1.850E-08	700	1781
GO:0016787	hydrolase activity	MOLECULAR_FUNCTION	1.918E-04	363	904
GO:0051536	iron-sulfur cluster binding	MOLECULAR_FUNCTION	2.151E-02	20	21
GO:0016020	membrane	CELLULAR_COMPONENT	6.407E-05	263	605
GO:0051540	metal cluster binding	MOLECULAR_FUNCTION	2.151E-02	20	21
GO:0046872	metal ion binding	MOLECULAR_FUNCTION	2.993E-02	275	722
GO:0006850	mitochondrial pyruvate transport	BIOLOGICAL_PROCESS	6.854E-03	328	853
GO:0006839	mitochondrial transport	BIOLOGICAL_PROCESS	3.365E-03	179	413
GO:0006807	nitrogen compound metabolic process	BIOLOGICAL_PROCESS	1.976E-03	548	1494
GO:0097159	organic cyclic compound binding	MOLECULAR_FUNCTION	1.850E-08	700	1781
GO:0097159	organic cyclic compound binding	MOLECULAR_FUNCTION	4.798E-02	79	168
GO:0071704	organic substance metabolic process	BIOLOGICAL_PROCESS	6.213E-07	695	1813
GO:0055114	oxidation-reduction process	BIOLOGICAL_PROCESS	3.011E-03	240	584
GO:0016684	oxidoreductase activity, acting on peroxide as acceptor	MOLECULAR_FUNCTION	3.249E-02	112	253
GO:0004601	peroxidase activity	MOLECULAR_FUNCTION	2.404E-02	57	105
GO:0016462	pyrophosphatase activity	MOLECULAR_FUNCTION	1.943E-03	106	211
GO:0030140	trans-Golgi network transport vesicle	CELLULAR_COMPONENT	4.263E-03	178	413
GO:0012510	trans-Golgi network transport vesicle membrane	CELLULAR_COMPONENT	6.854E-03	139	310
GO:0055085	transmembrane transport	BIOLOGICAL_PROCESS	2.009E-03	123	256
GO:0006810	transport	BIOLOGICAL_PROCESS	1.421E-04	214	477

Virulence and saprobic ability of fungal genotypes

In the virulence assay, *in vitro* mortality of pine germlings was recorded for a period of 40 days. Forty days post inoculation (DPI), each fungal genotype had killed all germlings it had been inoculated onto, but there was significant variability among genotypes in the average DPI needed to kill all germlings. Seven virulent genotypes (one pure *H. annosum* and six hybrids) were able to induce damping-off of germlings within 20 DPI (Suppl. material 4: Table S2). By contrast, three genotypes (one pure *H. irregulare* and two hybrids) killed germlings after 30 DPI. PCoA on

Table 3. Gene Ontology terms over-represented in the dataset of 1,418 introgressed *H. annosum* alleles in hybrid *H. irregulare* genotypes. FDR: False Discovery Rate (FDR) corrected p-value.

GO-ID	Term	Category	FDR	Sequences (N°) in Test Group	Sequences (N°) in Reference Group
GO:0008152	metabolic process	BIOLOGICAL_PROCESS	4.201E-10	824	3403
GO:0000166	nucleotide binding	MOLECULAR_FUNCTION	5.605E-07	258	886
GO:0032553	ribonucleotide binding	MOLECULAR_FUNCTION	1.131E-04	193	670
GO:0009987	cellular process	BIOLOGICAL_PROCESS	1.166E-04	576	2457
GO:0005488	Binding	MOLECULAR_FUNCTION	1.342E-04	641	2781
GO:0032555	purine ribonucleotide binding	MOLECULAR_FUNCTION	2.269E-04	184	646
GO:0035639	purine ribonucleoside triphosphate binding	MOLECULAR_FUNCTION	2.269E-04	183	641
GO:0017076	purine nucleotide binding	MOLECULAR_FUNCTION	2.961E-04	185	655
GO:0043167	ion binding	MOLECULAR_FUNCTION	4.752E-04	461	1942
GO:0005622	intracellular	CELLULAR_COMPONENT	7.746E-04	266	1033
GO:0005524	ATP binding	MOLECULAR_FUNCTION	8.246E-04	158	552
GO:0032559	adenyl ribonucleotide binding	MOLECULAR_FUNCTION	8.330E-04	159	557
GO:0010467	gene expression	BIOLOGICAL_PROCESS	9.074E-04	151	524
GO:0030554	adenyl nucleotide binding	MOLECULAR_FUNCTION	1.122E-03	160	566
GO:0043039	tRNA aminoacylation	BIOLOGICAL_PROCESS	1.529E-03	17	19
GO:0043038	amino acid activation	BIOLOGICAL_PROCESS	1.529E-03	17	19
GO:0016070	RNA metabolic process	BIOLOGICAL_PROCESS	2.027E-03	122	411
GO:0004812	aminoacyl-tRNA ligase activity	MOLECULAR_FUNCTION	2.537E-03	16	18
GO:0006418	tRNA aminoacylation for protein translation	BIOLOGICAL_PROCESS	2.537E-03	16	18
GO:0019787	ubiquitin-like protein transferase activity	MOLECULAR_FUNCTION	6.277E-03	10	7
GO:0017111	nucleoside-triphosphatase activity	MOLECULAR_FUNCTION	9.330E-03	75	235
GO:0034660	ncRNA metabolic process	BIOLOGICAL_PROCESS	2.207E-02	31	73
GO:0005634	nucleus	CELLULAR_COMPONENT	2.251E-02	122	447
GO:0006399	tRNA metabolic process	BIOLOGICAL_PROCESS	4.406E-02	24	53

virulence data differentiated two large groups, one including more virulent genotypes and one including less virulent genotypes (Suppl. material 3: Figure S11), however there was no clear correlation between group assignment and genomic background of the genotypes.

In the saprobic assay, the extent of *in vitro* mycelial growth on a wood-rich substrate was assessed for a period of ten days. Only one pure *H. irregulare* genotype (9OA) was able to fully colonize the Petri dishes within the time frame of the experiment, while other genotypes varied in the extent of their growth (Suppl. material 4: Table S3). PCoA on saprobic assay data differentiated genotypes based on their growth rate. Three *H. annosum* hybrids had a growth rate comparable to that of all pure *H. irregulare* genotypes. All the remaining hybrids and the *H. annosum* genotype were placed in a cluster of genotypes characterized by lower growth rates (Figure 5). Permutation tests determined that growth rates of *H. annosum* hybrids were significantly

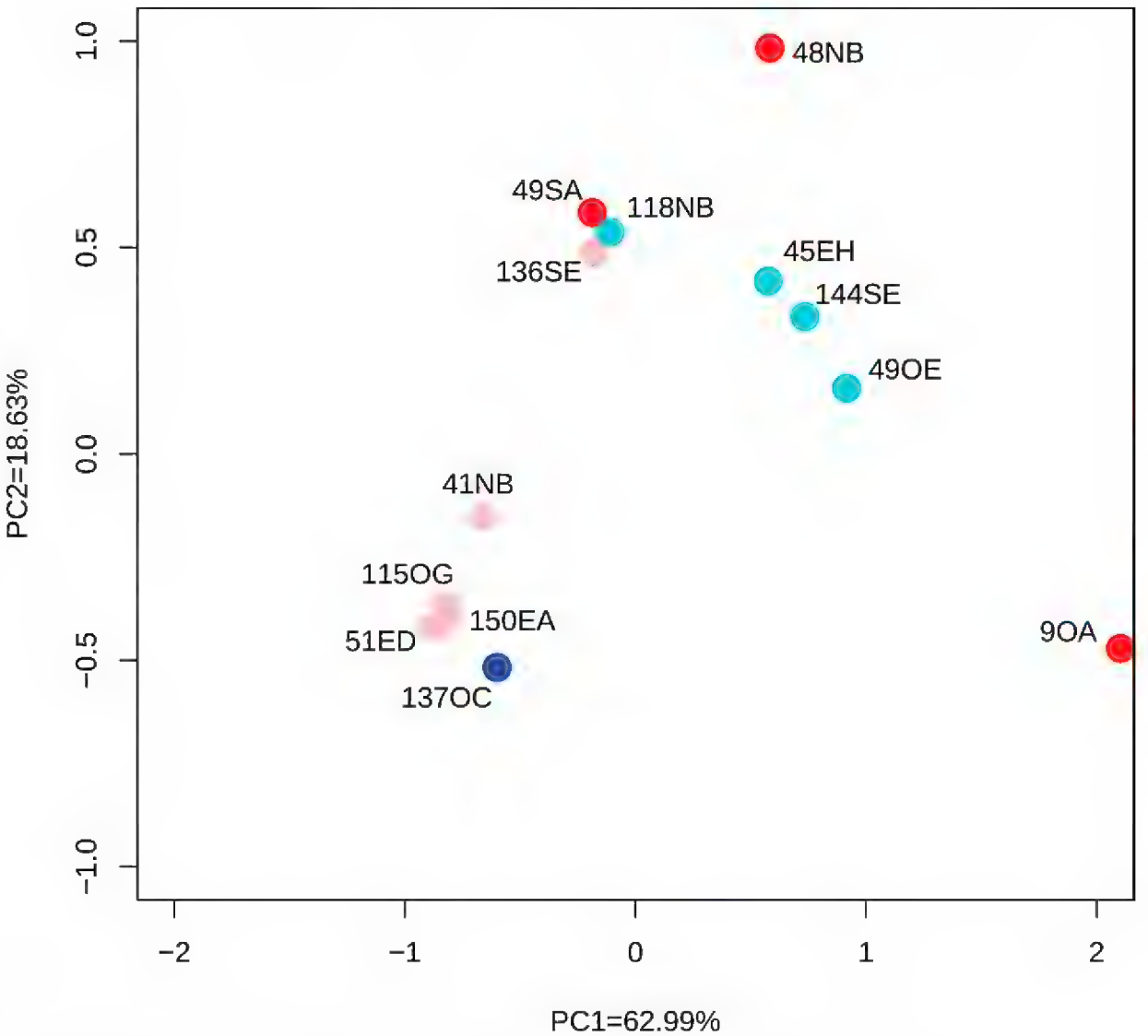

Figure 5. Results of PCoA on phenotypic data obtained from the saprobic assay. Saprobic ability increase from left to right. Red and blue dots represent pure *H. irregulare* and *H. annosum* genotypes, respectively. Pink and cyan dots represents hybrid genotypes assigned as *H. irregulare* and *H. annosum*, respectively.

higher than those of *H. irregulare* hybrids (observed value of the t-statistic = 6.506, p-value adjusted for multiple testing = 0.00043). The saprobic growth of *H. annosum* hybrids was no different from that of *H. irregulare* pure genotypes (p-value adjusted for multiple testing > 0.05). On the other hand, the growth rate of *H. irregulare* hybrids was significantly lower than that of pure *H. irregulare* genotypes (observed value of the t-statistic = -2.439, p-value adjusted for multiple testing = 0.000051).

Discussion

The introgression of both exotic genotypes and exotic alleles in native populations, where exotic is defined here as pertaining to an exotic species, is knowingly associated with biological invasions. During interspecific hybridization, the interspecific introgression of alleles bears significant evolutionary implications, shifting the focus from the more traditional "species-centric" to a "gene-centric" concept of invasion (Crispo et al. 2011). In this study, we have shed light on the dynamics of hybridization-mediated allelic introgression between an invasive and a native fungal species. We performed

the study by sampling pure and admixed genotypes in an area in Italy where the North American forest pathogen *H. irregulare* has been introduced and is hybridizing with the native Eurasian forest pathogen *H. annosum* (Gonthier et al. 2007; Gonthier and Garbelotto 2011). This system is particularly interesting as the precise date and location of introduction are known (Gonthier et al. 2004), the biology and population structure of pathogens are also known, the two species are fully interfertile, and 15 years of research have identified some of the likely mechanisms behind the clear demographic dominance of the exotic species compared to the native one.

For this study, we selected a set of 15 natural genotypes with levels of admixture ranging between zero (pure genotypes) and 45% (fully admixed) based on a large number of anonymous AFLP markers. The fact that all studied genotypes had successfully established themselves on wood in a natural setting suggested they were all sufficiently fit to survive in nature. Our expectation that these genotypes would display variably admixed genomic regions (Elgvin et al. 2017) and variable phenotypic fitness proved correct, allowing us to associate genomic variability and phenotypic fitness with specific introgression histories.

Phylogenomic analyses confirmed that the selected natural hybrids varied in the level of genomic admixture. Three hybrid genotypes (118NB, 150EA and 49OE) had a balanced level of admixture, suggesting either a recent hybridization event between two pure parental genotypes or balanced backcrosses with both parental species. The remaining six genotypes had signs of introgression in a relatively small portion of their genome, indicative of multiple backcrosses with a single parental species, to which they could be assigned. Some scaffolds (e.g. scaffolds 6, 12 and 14) showed significantly lower levels of introgression, suggesting these scaffolds contain genes regulating essential functions or species-specific genes (Olson et al. 2012).

The genomes of *H. irregulare* hybrids were profoundly affected by introgression from *H. annosum*, and only approximately 2 Mbp appeared to have been left untouched, while the remaining 30 Mbp were subject to allelic replacement. A significantly larger amount (about 7 Mbp) of the genome of *H. annosum* hybrids, instead, had been left untouched by the introgression of *H. irregulare* alleles, suggesting a smaller scale process in terms of genomic rearrangement. Regions not affected by alleles introgression might be subjected to intra locus recombination, however, in this study, these regions were estimated to be only approximately 24% and 7% of the genome in *H. annosum* and *H. irregulare,* respectively. The effects of intralocus recombination, despite their significance, are not treated in this work and these regions were excluded from the quantification of allelic introgression.

The size of blocks introgressed from the exotic *H. irregulare* into the native *H. annosum* was on average one order of magnitude larger than the size of those introgressed from *H. annosum* into *H. irregulare*. Small block dimensions (about 85 bp on average) and high number of genomic blocks (more than 14 000) introgressed into the exotic species may result in the excessive fragmentation of chromosomal blocks. As a result, exons may be partial, non-functional and may be more likely to be lost during recurrent recombination events (Sachdeva and Barton 2018). On the other hand, a low

number (about 6 000) of larger blocks (on average 890 bp in size) introgressed from *H. irregulare* may suggest that putatively adaptive and functional alleles may be truly integrated and preserved into the recipient *H. annosum* genomes. It is known that large genomic blocks have a better chance to be maintained through multiple generations when compared to small blocks (Baird et al. 2003; Sachdeva and Barton 2018).

Alleles contained in the larger blocks introgressed from *H. irregulare* into *H. annosum* showed a GO term enrichment related to catabolic pathways, i.e. peroxidases, heme binding proteins, oxidoreductases, metal ion binding proteins and to vesicle trafficking. Oxidoreductases and heme peroxidase genes have been identified among those that best differentiate the highly saprobic *H. irregulare* from the less saprobic *H. annosum* (Sillo et al. 2015), and some of them, e.g. manganese peroxidases, are known to play a role in lignin degradation during saprobic colonization of wood by *Heterobasidion* species (Yakovlev et al. 2013). All experimental evidence has indicated that higher saprobic potential explains much of the invasiveness of *H. irregulare* (Giordano et al. 2014; Giordano et al. 2019). Another category of over-represented GO terms among *H. irregulare* alleles introgressed into *H. annosum* was related to mitochondrial transport. Interestingly, this is another category of genes that well differentiates the two species at the genomic level (Sillo et al. 2015), with *H. irregulare* nuclear genes better at regulating mitochondrial functions in admixed genomes than *H. annosum* nuclear genes (Sillo et al. 2015). Based on the preexisting literature, the introgression of alleles belonging to these gene groups above into *H. annosum* had been predicted. Alleles involved in clathrin-mediated vesicular trafficking were also significantly enriched among alleles introgressed in *H. annosum* hybrids: this catabolic process plays a role in the breakdown of host defenses and in the development of polarized fungal hyphal growth (Steinberg 2007; Shultzhaus et al. 2017). To explain the advantages provided by the presence of these alleles, it should be noted that mutants of the fungus *Candida albicans* (C.P. Robin) Berkhout with modifications in genes affecting vesicular trafficking are plagued by defects in the cytoskeleton (Epp et al. 2010). Finally, alleles related to "iron-sulphur cluster binding" and "membrane" were found to be introgressed in *H. annosum*. Iron-sulphur cluster enzymes are known to play different roles in cells, including a role in regulating genomic integrity (Stehling et al. 2012) and in detoxification (Haas et al. 2008). Maintaining genomic integrity is obviously an important function in admixed hybrid genomes, while detoxification may be relevant, for example, during delignification processes (Sista Kameshwar and Qin 2020). Moreover, genes related to membrane may affect the activity of extracellular enzymatic activities, required by wood decay fungi to delignify the substrate in which they live (Wu et al. 2020).

Conversely, directional allelic introgression from *H. annosum* to *H. irregulare*, although larger in scale, was limited to DNA-methylation and transcription factor genes. Genes related to DNA-repair have been reported in hybridization events in animals, e.g. sparrows, and it has been hypothesized that they may be selected by nature to modulate epistatic interactions following genomic alterations caused by hybridization (Walsh et al. 2018). The re-patterning of DNA methylation has been also recently

documented in plants (Li et al. 2019). This phenomenon is a process affecting gene expression and silencing of mobile elements in the genome (Martienssen and Colot 2001). The introgression of these alleles can be regarded as the result of a selection of *H. annosum* alleles involved in epistatic regulation within the highly mosaic-like *H. irregulare* hybrid genotypes. In other words, given that the consequence of mosaic architecture of hybrid genomes can be the alteration of transcriptomic profiling and/or proliferation of mobile elements, methylation and regulation of genes may contribute to the stability of the hybrid genome. Regulatory genes may also be important in optimizing gene expression and adaptation in a microbial species facing a new environment (Elena and Lenski 2003), and it has been hypothesized that evolution may promote adaptations through already available and even acquired genetic tools rather than generating novel catabolic components (Lavoie et al. 2010). In addition, *H. annosum* GO terms involved in tRNA aminoacylation processes were identified as being overrepresented in *H. irregulare* hybrids. It is worth noting that tRNA aminoacylation is not only involved in protein translational fidelity that is indispensable for cell survival, but also allows for the tolerance of low levels of mistranslation, a predicament that may occur during stressful exposure to new environments (Wiltrout et al. 2012; Pan 2013). Thus, it could be inferred that the introgression of these alleles may be beneficial to *H. irregulare* hybrids constantly facing exposure to novel environments. A further transcriptomic analysis (i.e. RNA-sequencing) will be pivotal to clarify the functional role of introgressed alleles during different life phases of the hybrids (e.g. wood colonization, infection).

The sabrobic *in vitro* assay revealed that *H. annosum* hybrids containing *H. irregulare* alleles involved in saprobic decay had an increased saprobic ability, comparable to that of pure *H. irregulare* genotypes. Based on the results of the saprobic assay, it is reasonable to hypothesize these *H. annosum* hybrid genotypes may be competitive against pure *H. irregulare* genotypes. By contrast, *H. irregulare* hybrid genotypes showed a decrease in saprobic growth when compared to pure *H. irregulare* genotypes. We believe the impact of large-scale introgression is costly to hybrid *H. irregulare* genotypes, resulting in a reduction in fitness. We suggest that viability may be maintained only in those *H. irregulare* hybrids that: first, absorb transcription and methylation related genes from the native species stabilizing their genomic instability caused by large genomic alterations, and, second, maintain their alleles involved in production of peroxidase and heme-binding proteins, mitochondrial transport and vesicle trafficking. This last point is corroborated by the fact that the alleles involved in catabolic pathways were not affected by introgressive events, i.e. these alleles were never replaced by *H. annosum* alleles in any of the *H. irregulare* hybrids. Negative epistatic interactions and the breakdown of advantageous gene complexes (Lynch 1991; Guerrero et al. 2017) in *H. irregulare* recipient genomes, highly modified by large scale introgression events, are two plausible explanations for this detrimental effect on fitness.

Virulence assays in the past have not been able to differentiate the two species (Garbelotto et al. 2010; Pepori et al. 2019). The results of the virulence assay performed in

this study further confirmed that virulence may be a genotype-specific trait, rather than a species-level trait as previously suggested (Garbelotto et al. 2010). We note, though, that all hybrids fell within the range of virulence identified for the pure parental genotypes used in the study, and so all can be regarded as ecologically viable. This result confirms that by using genotypes that have established themselves on wood in a natural setting, we obtained a representative sample of functionally and ecologically viable genotypes. In addition, the result also confirms that hybrid swarms represent a viable genetic bridge for the transfer of genetic material between the two species (Brasier 2001).

Although we recognize that the number of genotypes characterized as pure *H. annosum* and *H. irregulare* used in our phenotypic tests is limited, the genotypes employed here represented a select sample representative of each species, and results were in agreement with our expectations. In a previous comparative genomic study, genes involved in virulence have been reported to be conserved between the two species (Sillo et al. 2015), resulting in indistinguishable virulent phenotypes (Garbelotto et al. 2010; Pepori et al. 2019). Conversely, genes involved in catabolic processes, including wood decay, and genes regulating nucleus-mitochondrion communication have been shown to diverge (Sillo et al. 2015) and to be associated with different phenotypes (Giordano et al. 2014; 2018). Results of this study are in agreement with a role of selection driving introgression: alleles from divergent loci such as those involved in catabolic processes were significantly enriched as a result of introgression and caused a measurable phenotypic change in the species receiving them by increasing its saprobic ability. On the other hand, we could not document introgression-related enrichment of genes directly involved in virulence of live plants and no measurable change in the associated phenotype could be measured.

It is known that competition with parental species living in the same environment may negatively affect the success of hybrids (Stukenbrock 2016). In the pathosystem studied here, the exotic *Heterobasidion* species may outcompete or even replace the native species, possibly due to its higher saprobic and sporulation potential (Gonthier et al. 2014). Our results suggest that *H. annosum* genotypes hybridizing with *H. irregulare* may become as competitive as those belonging to the exotic species, by receiving alleles that provide them with adaptive phenotypic advantages, including an increased saprobic wood decay potential. If this suggested scenario was correct, these alleles introgressed from *H. irregulare* into *H. annosum*, including those related to the saprobic ability among others, may move into *H. annosum* populations both inside and outside the hybrid zone. The rapid absorption of advantageous or adaptive exotic alleles through introgressive hybridization has been previously documented for many organisms (Fitzpatrick et al. 2010; Crispo et al. 2011) including the fungi (Hessenauer et al. 2020). Thus, *H. annosum* hybrids, with some specific *H. irregulare* alleles introgressed in their genome, may themselves represent a threat to the stability of European forests. On the other hand, introgression from the native into the invasive species affected a larger portion of the genome and involved alleles exclusively associated with gene regulation and transcription processes, resulting in lower saprobic growth, but ensuring the viability of hybrid genotypes.

Conclusion

Overall, our results show that mating events leading to viable and fertile *Heterobasidion* hybrids occur frequently in the Latium region of Italy where the exotic forest pathogen *H. irregulare* is now sympatric with the native forest pathogen *H.annosum*. The resulting allelic introgression has identified specific classes of adaptive *H. irregulare* alleles that confer an advantage to recipient native *H. annosum* genotypes. Given the phenotypic outcomes of these introgression events including a measurable increase in saprobic potential of *H. annosum* individuals, we propose an operational shift in the monitoring of the invasion process to include not only the detection of exotic *H. irregulare* genotypes, but also the presence of adaptive exotic *H. irregulare* alleles or genes spreading into native *H. annosum* populations. A gene centric approach to the study of invasions has been previously advocated (Petit, 2004) and clear adaptive implications of genic introgression between tree pathogens have been previously documented for the two fungi causing Dutch elm disease (Hessenauer et al. 2020).

In conclusion, despite the limited number of sequenced hybrid genotypes, this study suggests that horizontal allelic movement occurred as a result of interspecific hybridization, but it was qualitatively and quantitatively different when comparing the two directions of introgression. A large population genomics survey in and near the invasion area in Central Italy targeting introgressed alleles identified in this study will be pivotal to further validate these results.

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Sequence data were submitted to the EMBL database ENA - European Nucleotide Archive as a project under the PRJEB36378 accession number. All other data were available within the article or its supplementary materials.

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Supplementary material 1

Dataset S1. List of introgressed *H. irregulare* alleles

Authors: Fabiano Sillo, Matteo Garbelotto, Luana Giordano, Paolo Gonthier

Data type: dataset

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Supplementary material 2

Dataset S2. List of introgressed *H. annosum* alleles

Authors: Fabiano Sillo, Matteo Garbelotto, Luana Giordano, Paolo Gonthier

Data type: dataset

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Supplementary material 3

Figures S1–S11

Authors: Fabiano Sillo, Matteo Garbelotto, Luana Giordano, Paolo Gonthier

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Supplementary material 4

Tables S1–S4

Authors: Fabiano Sillo, Matteo Garbelotto, Luana Giordano, Paolo Gonthier

Data type: tables

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